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## Relationship of *Macrophoma* and *Diplodia*

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(WITH PLATE 25)

In December, 1902, Mr. Earle brought back to the New York Botanical Garden from Jamaica, West Indies, various collections of cocoanut affected with diseases. One set was handed over to the writer to see how it would develop when grown in cultures. It was *no. 510*, collected at Bowden, Jamaica, November 18, 1902, on flower-bud spathes of *Cocos nucifera*, labelled "dying of wasting disease." The spathes were covered with black spots just visible to the unaided eye, which proved to be pycnidia of *Macrophoma* and *Diplodia*, so closely associated that from one point both the hyaline unicellular *Macrophoma* spores and the brown two-celled *Diplodia* spores could be secured.

In March, 1903, cultures were started from the spathes by scraping off some of the black pycnidia where *Macrophoma* spores had previously been found, examining with a microscope and transferring a few spores to ordinary neutral agar. In the same manner cultures were taken from spathes where *Diplodia* spores had been seen. In this way two sets of cultures from each kind of spore were obtained, with reasonable assurances that they started one from pure *Macrophoma* spores and the other from pure *Diplodia*.

At first only agar and potato were used as media, then bread and milk, bread and water and pith and blade of cocoanut leaf were added. All the cultures were kept in a dark room where the temperature was uniformly at about 24°C. In five days or less after sowing spores or mycelium on agar, vigorous, spreading colonies of silky hyphae were evident. When they were still young, however, it was found best to transfer them to one of the other media; for this fungus will not develop well on agar alone, active growth ceasing in a week or ten days and a few dark chlamydospores being the only result; whereas on potato and the other materials the growth is vigorous and rapid from the begin-

ning. In spite of such good growth pycnidia develop slowly and sparingly on potato but very freely on cocoanut pith. It was not possible to obtain young healthy cocoanut plants which could be inoculated with pure *Macrophoma* in order to prove whether it was parasitic or simply saprophytic. When fresh leaves from a greenhouse were used for inoculation, moulds and other fungi which were already established quite prevented the *Macrophoma* from growing at all.

In the development of the fungus, first a white film of mycelium spreads quickly over most of the medium; in about a week parts become dark green and gradually black, and in ten days to two weeks from the sowing the pycnidia are formed. On cocoanut pith, bread or potato cultures these were often quite above the substratum, even as early as eight or nine days from the sowing, when they looked like tiny green bubbles covered with hyphae. Even in this immature condition the *Macrophoma* spores were abundant; being pale green, granular and often containing what appeared to be oil-drops, and seeming to have more abundant contents than those from maturer pycnidia. In a damp atmosphere the *Macrophoma* spores are apt to come out of the mouth of the pycnidia and form a white mass. No spores which resembled conidiospores were noticed, but there were several other forms which seemed to be sclerotial or resting in their function. Soon after the green color came in a culture, on examining the mycelium it would be seen that a few cells or as many as eight in a hypha had become round or oblong, thick-walled, brown, sometimes quite rough,  $25-29\mu \times 8-18\mu$ . They germinate very readily, putting out several tubes from one spore (FIGURE 4). Sometimes two of these will cling together and the two might easily be mistaken for a *Diplodia* spore. Around the outside of the pycnidium there is apt to be a mass of empty two-celled bodies, one cell being slightly smaller than the other, as in a *Puccinia* teleutospore. Possibly they are merely short, swollen hyphae similar to the cells which make the outside wall of the pycnidium.

From the time the cultures were first obtained pure it was evident that the growths of *Macrophoma* and *Diplodia* were very much alike, as had been suspected from their close association and the

similarity of their pycnidia. So it was with no little pleasure that pycnidia were noticed on two separate cultures, one pure *Macrophoma* and the other pure *Diplodia*. Cultures on cocoanut pith started January 11th showed on January 23d pycnidia with an abundance of *Macrophoma* spores in each of the cultures, and on February 12th there was obtained from the same pycnidium both unicellular hyaline *Macrophoma* spores and bicellular brown *Diplodia* spores. This is the average time that it takes a pure culture on the most favorable medium to develop, namely 10–12 days for pycnidia with *Macrophoma* spores and 12–18 days more before *Diplodia* spores are also abundant. There may be a slightly earlier development of the two-celled spores in the pycnidia of cultures from *Diplodia* spores than those from *Macrophoma*, but in general there is no difference in appearance of mycelium, size, or shape of spores or pycnidia. There certainly seems to be no doubt that the unicellular white *Macrophoma* spores in the pycnidia are simply the immature forerunners of the mature *Diplodia* spores.

In microtome sections of the leaf with the fungus growing on it from a culture five weeks old, the cells near the pycnidium seem much disorganized by the intercellular hyphal threads, being contracted into irregular darkly-stained masses and the cell-walls being difficult to trace. This affected area extends along the lower part of the leaf to some distance on either side of the pycnidium, but does not go through to the upper side.

Material for microtome sections was put into weak Flemming's solution to kill and fix. It was then washed in water, dehydrated, and imbedded in paraffin. Some sections were mounted in Canada balsam without any staining, but the *Macrophoma* spores proved to be almost invisible and some of the *Diplodia* spores too dark. As the pycnidia are very black an attempt was made to decolorize the sections by putting them into hydrogen peroxide and alcohol for about five hours. After washing they were stained in saffranin, gentian violet and orange gentian and mounted in balsam. This combination stains the *Macrophoma* spores orange and the rest of the pycnidium brown, but care must be taken not to overstain. The most satisfactory staining method was saffranin ten minutes, Delafield's hematoxylin five minutes, washing out excess of stain with acidified alcohol, and mounting in balsam. In

this way the *Macrophoma* spores are stained light purple and the rest of the fungus brown, while the leaf-tissues become brown or red.

The *Macrophoma* form of this species was described originally by Cooke in Grevillea (5: 101), under the name *Sphaeropsis palmarum*, as follows :

"Erumpens. Peritheciis subglobosis, applanatis, demum superne detectis, atris; sporis ellipticis, hyalinis, intus granulosis. On petioles and midribs of *Cocos nucifera*. Demerara. Spores  $.02 \times .012$  mm. Perithecia rather large, splitting the cuticle."

On the next page of the same paper Cooke thus describes the *Diplodia* form under the name *Diplodia epicocos* :

"Sparsa vel subgregaria. Peritheciis demum superficialibus vel semi-immersis, globosis, atris; sporis ellipticis, uniseptatis, constrictis, brunneis. On dead young leaves of *Cocos nucifera*. Spores  $.022 \times .01-.012$  mm. Externally resembling a small *Sphaeria* of the section *Denudatae*."

In the following description I have included both stages :

Pycnidia buried to erumpent, sometimes becoming superficial, black, carbonaceous, opaque, cells indistinct,  $150-250 \mu$  in diameter, subgregarious, globose or conical at the mouth; *Macrophoma* spores elliptical or ovoid, obtuse, hyaline, granular,  $20-25 \times 10 \mu$ . *Diplodia* spores elliptical, one septate, brown, obtuse, sometimes slightly constricted,  $20-25 \times 10 \mu$ ; spores borne on simple hyaline sporophores  $12-15$  by  $2-3 \mu$ , hyaline and brown spores in the same way, without any order; mycelium of contorted, irregular, septate threads, brown near the pycnidia and when old.

#### Explanation of plate 25

Fig. 1. Three pycnidia breaking through the epidermis of cocoanut spathe. Seen from above.  $\times 44$ .

Fig. 2. The single pycnidium from Fig. 1.  $\times 44$ .

Fig. 3. *Macrophoma* and *Diplodia* spores.  $\times 280$ .

Fig. 4. Chlamydospores sprouting.  $\times 192$ .

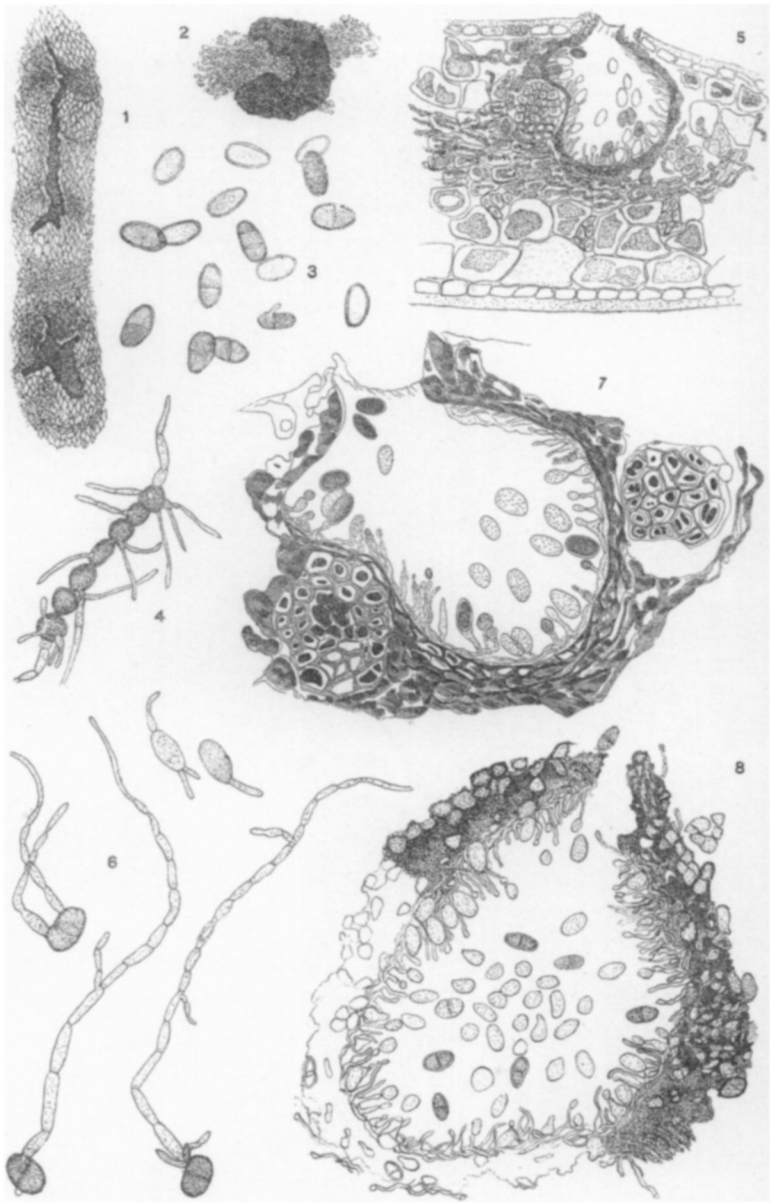
Fig. 5. Longitudinal section through pycnidium in leaf.  $\times 192$ .

Fig. 6. *Macrophoma* and *Diplodia* spores from a hanging drop culture. 48 hours old.  $\times 280$ .

Fig. 7. Similar to Fig. 5, more highly magnified and somewhat diagrammatic.  $\times 280$ .

Fig. 8. Longitudinal section of pycnidium growing above medium.  $\times 280$ .

Figs. 3, 6 and 7 are drawn with a camera lucida.



MACROPHLOMA AND DIPLODIA